IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	10/696,671
Applicant	:	Ivarie et al
Filed	:	October 28, 2003
Title	:	Transgenic Avians That Lay Eggs Containing Exogenous Proteins (amended)
TC/A.U.	:	1633
Examiner	:	Kaushal, Sumesh
Docket No.	:	AVI-000CON

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Date August 13, 2007
Signature
Name Kyle Yesland

Honorable Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DR. ROBERT D. IVARIE PURSUANT TO 37 CFR 1.132 (IVARIE DECLARATION)

Sir:

I, Dr. Robert D. Ivarie, hereby declare as follows:

1. I currently hold the position of Professor and Head of Genetics, University of Georgia. My professional experience and educational background are detailed in my attached curriculum vitae (IVARIE CURRICULUM VITAE).

2. As a co-inventor, I have personal knowledge of the invention disclosed and claimed in the above-referenced patent application (hereinafter the "Application").

3. I understand that the Patent Examiner in the subject case has rejected certain claims based on the premise that making germ-line transgenic avians which produce exogenous protein in the oviduct is unpredictable and not routine and that the experimentation required to do so is undue.

4. The Application as originally filed was sufficient at the time of filing to enable a practitioner of ordinary skill in the art to produce a wide variety of exogenous proteins in the oviduct of germline transgenic avians on a routine basis. In fact, the methods disclosed in the Application have proven to be robust and reliable enabling us to successfully make germline transgenic birds which lay eggs containing a number of proteins specifically named in the Application (for example, at page 31 of the Application) including: β-lactamase, granulocyte colony stimulating factor (G-CSF), erythropoietin (EPO) and interferon, i.e., interferon alpha 2 (IFNα2). Furthermore, by following the disclosure of the Application, a practitioner of ordinary skill in the art would be able to make lines of germline transgenic avians that lay eggs containing many different proteins in addition to those proteins that have been produced thus far and in addition to those proteins disclosed in the Application.

5. Germline transgenic chickens that produce beta lactamase (BL) in their oviduct were made in accordance with the Application. The data for the production of BL germline transgenic birds is as follows:

Production of G0 chimeric germline transgenic NLB-CMV-BL chickens was described in Examples 1 to 3 of the Application. G1 germline transgenic birds that produce BL in the oviduct were produced from the chimeric germline transgenic G0 male bird having the highest level of transgene in the sperm using standard breeding methodologies apparent to practitioners of ordinary skill in the art, i.e., the G0 roosters containing transgene in their sperm were crossed with non-transgenic chickens. Out of a total of 1026 G1 offspring tested by PCR analysis of genomic DNA, one rooster and two hens tested positive for the NLB-CMV-BL transgene. Eggs laid by the G1 germline transgenic females and their descendents contained between about 0.5 μg/ml and about 1.6 μg/ml of

BL, as determined by ELISA.

6. The methods used for making germline transgenic avians that produce G-CSF, EPO and interferon are disclosed in the Application. That is, the NLB-CMV-BL vector of Example 1 in the Application was altered to replace the BL coding sequence of the vector with the coding sequences for G-CSF, EPO and IFNα2. Germline transgenic birds were obtained using these modified NLB-CMV vectors in accordance with methods disclosed in the Application. These results are discussed in the following paragraphs.

7. Germline transgenic chickens that produce IFNα2 in their oviduct were made in accordance with the Application. The IFNα2 coding sequence was optimized for chicken codon usage, though such codon modification is not required to obtain useful yield of exogenous protein from the egg white as can be seen in the production of other proteins described herein. The description for the production of IFNα2 germline transgenic chickens is as follows:

The BL coding sequence of NLB-CMV-BL was replaced with the IFNα2 coding sequence optimized for chicken codon usage, producing NLB-CMV-IFNα2. NLB-CMV-IFNα2 transduction particles were produced essentially as described in Example 2 of the Application. 300 White Leghorn chicken eggs were windowed and injected with the transduction particles essentially as described in Example 3 of the Application. Three chimeric germline transgenic G0 roosters with the highest NLB-CMV-IFNα2 transgene level in their sperm were bred to non-transgenic females by artificial insemination to produce G1 birds. The 1,597th G1 offspring tested by PCR analysis of genomic DNA was a germline transgenic male carrying the NLB-CMV-IFNα2 transgene. The male G1 offspring was bred to non-transgenic female chickens by artificial insemination to produce G2 offspring. Egg white from eggs laid by G2 germline transgenic females and their descendents contained on average about 2.7 μg/ml of IFNα2, as determined by ELISA. Purified IFNα2 obtained from eggs of the G2 birds and their descendents has entered clinical trials for FDA regulatory approval. Purification of exogenous proteins such as IFNα2 from eggs laid by transgenic birds can readily be accomplished by a practitioner of ordinary skill in the art using standard protein purification methodologies.

8. Germline transgenic chickens that produce G-CSF in their oviduct were made in accordance with the Application. The G-CSF coding sequence used was the human G-CSF nucleotide coding sequence. The description for the production of G-CSF germline transgenic chickens is as follows:

The IFNα2 coding sequence of NLB-CMV-IFNα2 was replaced with the human G-CSF coding sequence producing NLB-CMV-G-CSF. NLB-CMV-G-CSF transduction particles were produced essentially as described in Example 2 of the Application. 274 White Leghorn chicken eggs were windowed and injected with the transduction particles essentially as described in Example 3 of the Application. 41 of the eggs hatched. Two chimeric germline transgenic G0 roosters positive for the NLB-CMV-G-CSF transgene were bred to non-transgenic females by artificial insemination producing 4353 offspring, 14 of which were identified as germline transgenic G1's carrying the NLB-CMV-G-CSF transgene. Egg white of eggs laid by the G1 germline transgenic females and their descendents contained an average of about 3 μg/ml of G-CSF, as determined by ELISA. Purified G-CSF obtained from eggs of these G1 birds and their descendents has entered clinical trials for FDA regulatory approval. Purification of exogenous proteins such as G-CSF from eggs laid by transgenic birds can readily be accomplished by a practitioner of ordinary skill in the art using standard protein purification methodologies.

9. Germline transgenic chickens that produce EPO in their oviduct were made in accordance with the Application. The EPO coding sequence used was the human EPO nucleotide coding sequence. The description for the production of EPO germline transgenic chickens is as follows:

The IFNα2 coding sequence of NLB-CMV-IFNα2 was replaced with the human EPO coding sequence producing NLB-CMV-EPO. NLB-CMV-EPO transduction particles were produced essentially as described in Example 2 of the Application. 1234 White Leghorn chicken eggs were windowed and injected with the transduction particles essentially as described in Example 3 of the Application. 334 of the eggs hatched. Seven of the hatched G0 roosters tested positive for the NLB-CMV-EPO transgene.

Three chimeric germline transgenic roosters that tested positive for the NLB-CMV-EPO transgene were bred to non-transgenic females by artificial insemination to produce 1190 offspring, 14 of which were transgene positive germline transgenic G1's. Egg white of eggs laid by the G1 germline transgenic females or their descendents contained about 0.4 to 1.9 μg/ml of EPO, as determined by ELISA. Purification of exogenous proteins such as EPO from eggs laid by transgenic birds can readily be accomplished by a practitioner of ordinary skill in the art using standard protein purification methodologies and, in fact, transgenic chicken derived EPO has been purified from eggs for use in *in vivo* and *in vitro* erythropoietin activity studies.

10. From the proceeding paragraphs it can be seen that production of transgenic birds that produce exogenous proteins in the oviduct is predictable and routine when following the teachings of the Application. As is expected a number of transgenic birds typically need to be screened in order to identify the transgenic G1 offspring (first generation of fully transgenic germline birds) obtained from the germline chimeras. However, such screening and identification can be accomplished routinely and with predictability by skilled technicians in the field of poultry science and molecular biology. In addition, identifying lines of G1 birds which lay eggs containing useful quantities of the transgene encoded exogenous protein has been predictable and routine using vectors of the invention. For example, use of the non-tissue specific CMV promoter to express the exogenous protein in the avian oviduct has been routine and has not required undue experimentation. In addition, random integration of the NLB vector into the avian genome has not made practicing the invention unpredictable and has not imposed undue experimentation in order to practice the invention.

11. Approximately 50% of offspring produced by crossing non-transgenic birds with the G1 germline transgenic avians produced in accordance with the Application (and having a confirmed single transgene copy in their genome) were transgene positive. This inheritance pattern is what is expected based upon Mendelian inheritance, thus providing further confirmation of germline transmission originating from the germline chimeric birds. Furthermore, approximately half of all subsequent

offspring (G3, G4, G5, ect) obtained from the germline transgenic avian lines produced in accordance with the Application have been fully germline transgenic, as would be expected in stable germline transmission of a hemizygous allele.

12. In addition to the production of germline transgenic chickens, I believe that the vectors and methods described in the Application can be used to produce germline transgenic avians other than chickens. In particular, the infectivity of ALV is not limited to chickens. In support of this, provided below is data showing production of transgenic quails that were produced using the NLB-CMV-G-CSF retroviral vector of paragraph 8 above and methods described in the Application.

13. Fertilized quail eggs were windowed and injected with NLB-CMV-G-CSF transduction particles essentially as described in Example 3 of the Application and approximately 24% of the hatched G0 hens were positive for the NLB-CMV-G-CSF transgene. Eggs of the G0 transgenic quail hens contained between about 25 pg and about 160 pg of G-CSF as determined by ELISA of the egg white from eggs laid by the birds. A low yield of exogenous protein in the egg white is not unexpected in eggs of G0 avians since the birds will be chimeric for the transgene (i.e., only a small percentage of the cells in the G0 birds will be transgene positive).

14. The reason for producing these transgenic quail was for purposes related specifically to quantification of promoter activity, which can be accomplished in G0 chimeric birds. The reason to use quail for this purpose is the rapid maturation rate (time from hatch to egg laying) of quail compared to chickens, 6 weeks for quail opposed to 20-22 weeks for chickens. The transgenic quail were not made with the goal of producing a germline transgenic flock to produce exogenous proteins because the volume of egg white contained in a quail egg is quite small compared to that of a chicken. Therefore, the laborious task of screening for germline transgenic quail was not undertaken. However, I believe with a high level of certainty that G1 germline transgenic quail which produce exogenous protein in the oviduct could be obtained from the transgenic G0 quails that were produced. In addition, I believe germline transgenic

avians other than chicken and quail which produce exogenous protein in the oviduct can be produced in accordance with the invention as disclosed in the Application.

15. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the Application or any patent issuing thereon.

Signed _____
Robert D. Ivaric, Ph.D.

Dated 8/10/07